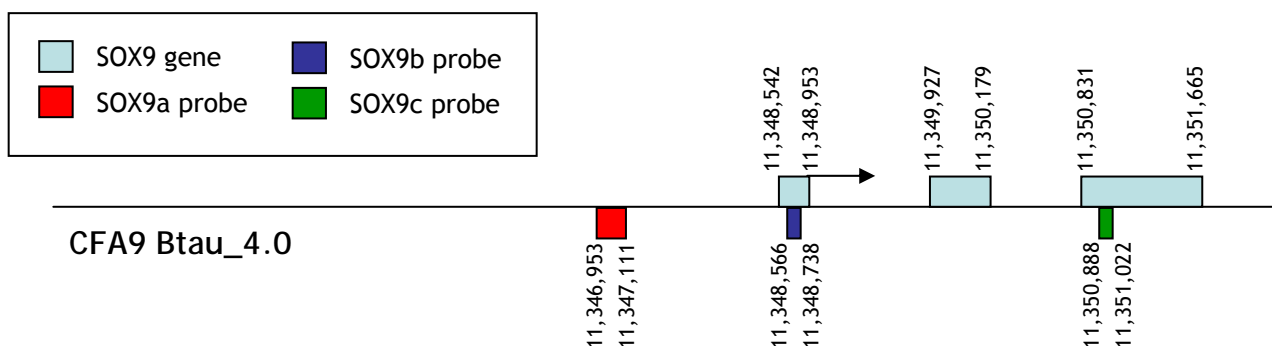


Exclusion of Sox9 gene duplication as responsible for the observed phenotype

In order to exclude *SOX9* genomic duplication as responsible for the phenotype observed a real time PCR approach were used.

Three different regions, surrounding the *SOX9* (NM_001002978) gene were analyzed by Real Time PCR and normalized with a PCR amplification involving *GAPDH* gene.

The position of the *SOX9* gene as well as of the three probes are reported below.



Primers used in Real Time PCR amplification and PCR product s size

Sox9a probe	5'-GGAAACACCCGACTTACA-3'	159 bp
	5'-GTCACTCGGGTTGCAGAAAG-3'	
Sox9b probe	5'-GAAGATGACCGACGAGCAG-3'	173 bp
	5'-TCCTCGCTCTCCTTCTTCAG-3'	
Sox9c probe	5'-GAATCGTGTGACCAGTTTGCT-3'	135 bp
	5'-TCCCCTCAAATGGTAATGAA-3'	
GAPDH probe	5'-CAAGCTCAAAGGGCAAGAGT-3'	124 bp
	5'-GCAAACCTGGAACCGTGAGAT-3'	

6 DNA samples, obtained from 6 unrelated normal (fertile) dogs were used as control. These samples, as well as the DNA obtained from the two dog reported in this paper, were used to produce 3 independent replicates.

Sox9a, b and c probes amplification level were calculated following the formula:

$$\text{Exp} = \frac{1}{2^{\Delta \text{Ct}}} \quad \text{where } \Delta \text{Ct} = \text{Ct SOXa,b or c} - \text{Ct GAPDH}$$

The presence of a deletion, or duplication, were evaluated using the formula:

$$r = \frac{\text{Exp SOX9a, b or c}}{\text{Exp GAPDH}}$$

r=1 indicates the absence of genomic abnormalities
r=1.5 indicates the presence of an heterozygous duplication

RESULTS

Average Ct	<u>GAPDH</u>	<u>SOX9 A</u>	<u>SOX9 B</u>	<u>SOX9 C</u>
dog a	21,64	22,22	26,42	24,27
dog b	21,73	22,49	26,72	24,25
dog 1	21,24	22,70	26,40	23,44
dog 3	22,23	22,51	27,75	25,09
dog 4	22,54	22,35	27,45	24,87
dog 5	21,77	22,13	26,97	23,80
dog 9	20,76	22,16	25,98	25,27
dog 10	20,74	22,23	24,75	22,69

dog a = mixed breed Pitbull
 dog b = Cocker Spaniel
 dog 1, 3, 4, 5, 9, 10 = Control dogs

	<u>SOX9 A</u>	<u>SOX9 B</u>	<u>SOX9 C</u>
Exp= $\frac{1}{2^{\Delta Ct}}$	dog a 0,672062	0,036398	0,161544
	dog b 0,589134	0,031322	0,173941
	dog 1 0,363493	0,028067	0,218393
	dog 3 0,820742	0,021717	0,137262
	dog 4 1,140764	0,033262	0,198884
	dog 5 0,779165	0,027111	0,244008
	dog 9 0,377618	0,02683	0,043889
	dog 10 0,356013	0,062068	0,258816

dog a = mixed breed Pitbull
 dog b = Cocker Spaniel
 dog 1, 3, 4, 5, 9, 10 = Control dogs

	<u>SOX9 A</u>	<u>SOX9 B</u>	<u>SOX9 C</u>
Exp= $\frac{1}{2^{\Delta Ct}}$	dog a 0,672	0,036	0,162
	dog b 0,589	0,031	0,174
	average control dogs 0,640	0,033	0,184

dog a = mixed breed Pitbull
 dog b = Cocker Spaniel

	<u>SOX9 A / GAPDH</u>	<u>SOX9 B /GAPDH</u>	<u>SOX9 C /GAPDH</u>
dog a	1,05	1,10	0,88
dog b	0,92	0,94	0,95

These data clearly indicates the absence of genomic duplication involving SOX9 gene